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Gene transfer in *Nicotiana rustica* by means of irradiated pollen IV. Qualitative characters

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The inheritance of three qualitative characters is examined in the first and second generations derived from crosses, both within and between inbred lines of *Nicotiana rustica*, that were produced using pollen irradiated with 20 Krad of γ-rays. In the M_1 generation the radiation induced the mutational loss of dominant alleles for black ovaries and green flowers. In the subsequent M_2 and backcross generations significant disturbances were observed in the segregations of these characters in favour of the original maternal alleles. The average frequency of transmission of a maternal allele from the M_1 is estimated as 0·55, indicating mild selection against radiation damage in the M_1 gametophytes or in the resultant zygotes. Selection of this intensity is insufficient to explain the maternal trends previously reported in this species and is insufficient to be of value to plant breeding. Similar results from various species have been obtained by other workers with the exception of Powell *et al.* (1983), who present evidence of strong selection in *Hordeum vulgare*.

INTRODUCTION

Previous papers in this series (Werner *et al.*, 1984; Werner and Cornish, 1984) have described the karyotypes of the first (M_1) and second (M_2 and backcross) generation of an intervarietal cross of *Nicotiana rustica* using pollen heavily irradiated with gamma-rays. The M_1 generation was found to possess induced numerical and structural rearrangements of the chromosomes, which were inherited by the subsequent generation. From these results it was estimated that 80 per cent of the M_2 *N. rustica* plants examined by Caligari *et al.* (1981) contained similar chromosomal aberrations and it was proposed that at least part of the maternal trends present in their material could have resulted from the expression of mutational damage. These cytogenetical studies also showed that some aberrant chromosomes of paternal origin were transmitted less frequently to the second generation than their undamaged homologues. Selection of this kind in favour of chromosomes of maternal origin, whether acting in the M_1 gametophytes or in the M_2 zygotes, may also lead to a maternal M_2 generation as was proposed by Snape *et al.* (1983).

The relative importance of mutational loss and selection in determining the M_2 phenotype can be deduced only from a comparison of M_2 generations derived from reciprocal crosses. With this aim in mind the inheritance of three qualitative characters were examined in generations derived from irradiated pollinations of the two varieties of *N. rustica* used by Caligari *et al.* (loc. cit). The findings of this study are reported here.

MATERIALS AND METHODS

The two inbred varieties of *N. rustica*, V_{12} and V_{27} differ for three characters under major gene control; ovary colour is controlled by a single gene with black dominant to non-black, flower colour is controlled by two duplicate genes with green dominant to yellow (Dunkin, pers. comm.) and inflorescence morphology is probably controlled by three duplicate genes with the loose form dominant to the mophead form (Al-Banna, 1983). The inflorescence character is not expressed in a poor environment and so can only be reliably assessed on field-grown plants. V_{27} possesses the recessive alleles for all three characters having non-black ovaries, yellow flowers and a mophead inflorescence. The six loci are unlinked (Werner, 1984).

Pollen from both varieties was irradiated with 20 Krads of gamma-radiation and used to pollinate

Table 1 The four crosses with pollen irradiated at 20 Krad

Treatment	Original cross	No. of viable seed per capsule*	No. of M_1 plants	Mean no. of seed per capsule: M_1 selfed (M_2)	$V_{27} \times M_1$ (BC)	$M_1 \times V_{27}$ (RBC)	No. of families in field experiment: M_2	BC	RBC	No. of M_1 plants represented by all three kinds of family
20A	$V_{27} \times V_{27}$	1·1	18	8·3	36·3	3·5	4	3	3	2
20B	$V_{27} \times V_{12}$	35·3	256	28·4	46·5	70·5	12	24	24	12
20C	$V_{12} \times V_{27}$	11·6	93	23·3	12·0	43·5	19	10	16	10
20D	$V_{12} \times V_{12}$	66·8	211	61·3	81·7	61·3	7	14	14	7

* Normal crosses give between 300 and 600 viable seeds per capsule.

stigmas of both varieties to provide the reciprocal crosses and the selfs of both parents. Unirradiated pollinations were also made for comparison. Table 1 shows for each treatment the number of viable seed obtained per capsule and the number of M_1 plants raised in the glasshouse. Irradiated V_{27} pollen produced less seed than irradiated V_{12} pollen so that fewer plants were obtained from treatments A and C. The ovary colours and flowers of all the M_1 plants that flowered were assessed. With the exception of a few late flowering plants all M_1 plants were selfed. In addition, samples chosen to cover the range of germination rates were also backcrossed reciprocally to V_{27}; a minimum of three pollinations were made in each direction ($V_{27} \times M_1 = BC$, $M_1 \times V_{27} = RBC$).

The fertility of the M_1 plants was greatly reduced so that only a limited number of plants produced enough seed (more than 40) to contribute all three families to the field trial of the next generation (table 1). M_1 plants which contributed only one or two families were therefore also included. Twenty replicate plants of each family were grown in the field in two randomised blocks, although the number of replicates in some families was reduced by poor germination. In addition 75 plants were raised in the glasshouse for each of 11 BC and RBC families from the 20B treatment for additional assessment of the two colour characters.

M_1 RESULTS

Nine plants from the 20A treatment and 36 from the 20C treatment differed markedly from the other M_1 plants by being vigorous, fully fertile and completely maternal in appearance. These plants were probably self (unirradiated) contaminants and can all be traced to three possible contamination events. Progeny from a sample of both groups were included in the randomised field experiments and were found to be identical to their respective maternal parents for all qualitative and quantitative characters. These plants have, therefore, been excluded from further consideration.

In general the 20A and 20D M_1 plants closely resembled their respective parents, V_{27} and V_{12}, whilst the 20B and 20C M_1 progenies resembled the F_1 hybrid. The M_1 plants were, however, more variable than their unirradiated counterparts as a result of a range of morphological aberrations induced by the radiation. 23 per cent of the M_1's failed to flower. V_{27} pollen appeared more sensitive to the irradiation treatment giving fewer viable seed per pollination and a higher frequency of aberrant M_1 plants than V_{12} pollen. All the M_1 plants that flowered possessed the same flower colour and ovary colour as the comparable unirradiated plants except for some plants from the 20B treatment; of the 181 20B M_1 plants that flowered 38 had non-black ovaries and two had yellow flowers. There were also three green/yellow mosaic plants in this group. Thus 21 per cent of these plants had failed to inherit a functional allele for ovary colour from V_{12} and 1·1 per cent had failed to inherit either of the paternal alleles for flower colour. The probabilities of the black and the green alleles being lost by mutation can, therefore, be estimated as 0·210 and 0·105, respectively.

M_2 AND BACKCROSS RESULTS

The segregation ratios of the control and irradiated generations for all three characters are given in table 2. The ratios of the control generations were consistent with the genetic models described above. The segregations within the 20B and 20C M_2 and backcross generations were, therefore, tested against the expected ratios of 3:1 and 1:1 for ovary colour, 15:1 and 3:1 for flower colour and 63:1 and 7:1 for infloresence form. With a

Table 2 Second generation segregation ratios. Asterisks indicate significant departures from the expected ratios at the 5 per cent (*), 1 per cent (**) and 0·1 per cent (***) levels

		No. of families	Ovary colour B	NB	B:NB	χ^2	Flower colour G	Y	G:Y	χ^2	Inflorescence morphology NM	M	NM:M	χ^2
	F_2	—	118	42	2·81:1	ns	152	8	19:1	ns	158	2	79:1	ns
	B_1	—	117	121	0·97:1	ns	172	66	2·61:1	ns	140	28	5·0:1	ns
20A	M_2	4	0	64	—	—	0	64	—	—	16	47	1:2·94	—
20A	BC	3	0	50	—	—	0	50	—	—	1	48	1:48	—
20A	RBC	3	0	40	—	—	0	40	—	—	3	35	1:11·67	—
20B	M_2	12	141	93	1·52:1	***	220	14	15·71:1	ns	221	4	55·25:1	ns
20B	M_2‡	8	141	54	2·61:1	ns	—	—	—	—	—	—	—	—
20B	BC	24	493	792	0·62:1	***	941	344	2·74:1	ns	369	91	4·05:1	***
20B	BC‡	20	493	543	0·91:1	ns	—	—	—	—	—	—	—	—
20B	RBC	24	444	820	0·54:1	***	942	328	2·87:1	ns	375	87	4·31:1	***
20B	RBC‡	20	444	573	0·77:1	***	—	—	—	—	—	—	—	—
20C	M_2	19	272	70	3·89:1	ns	333	9	37:1	**	332	2	166:1	ns
20C	BC	10	113	59	1·92:1	***	141	31	4·55:1	*	143	29	4·93:1	ns
20C	RBC	16	154	153	1·01:1	ns	253	54	4·69:1	**	260	45	5·78:1	ns
20D	M_2	7	137	0	—	—	136	0	—	—	137	0	—	—
20D	BC	14	278	0	—	—	278	0	—	—	277	0	—	—
20D	RBC	14	264	(3)†	—	—	267	0	—	—	266	0	—	—

† Attributable to functional loss of the black allele.
‡ Families not segregating for ovary colour have been omitted.

single exception that will be discussed below all M_1 plants with non-black ovaries gave non-black progenies. The ovary colour data for the generations involved are presented both with and without the inclusion of these non-segregating families. The two yellow flowered 20B M_1 plants were insufficiently fertile to contribute progeny to the field experiment.

Significantly fewer than expected black ovaried plants were found in the three generations derived from the 20B M_1 plants. Removal of the non-segregating families, however, reduced two of the deviations to a non-significant level. Clearly, these two generations show the effects of mutational loss whilst the residual deviation in the RBC family can be ascribed to the effects of maternal selection in the pollen. In the families derived from the 20C treatment selection against the non-black, paternal allele is evident in the segregations of the BC generation. Similar deviations in favour of the maternal alleles can also be seen in the data on flower colour and inflorescence morphology, although the latter character must be interpreted with caution since the mophead form is poorly expressed in plants of reduced vigour, as the 20A data show.

The progeny of the 20D M_1 plants also provide evidence of both mutational loss and selection (table 2); a total of three non-black ovaried plants was obtained from two of the 20D RBC families. They can be attributed to mutational loss of the dominant black allele in two of the 14 20D M_1 plants used. The recovery of so few non-black plants in these two families and their absence from the equivalent BC families indicates that strong selection occurred in the M_1 gametophytes and/or the resulting zygotes against the induced damage.

The magnitudes of the deviations induced in the segregations of the two colour characters can be compared by estimating for each a parameter q, defined as the frequency with which alleles of maternal origin are transmitted from the M_1 to the second generation. Thus, for a single gene, the frequency of the recessive phenotype in the BC and RBC generations is q when the recessive allele is maternal and $(1-q)$ when the dominant allele is maternal. The M_2 generations provide estimates of q^2 and $(1-q)^2$ in these two cases. A two gene character provides estimates in a similar fashion. Table 3 shows the estimates obtained from the present data. (Families not segregating for ovary colour have been excluded.) It can be seen that the frequencies of the maternal alleles are greater than 0·5 in almost all cases regardless of the direction of the cross, and that the M_2 and back-crosses provide similar estimates. The average maternal frequency is 0·55; this indicates mild selection only.

The segregation data presented in table 2 conceals differences between families within each

Table 3 Estimates of q, the proportion of alleles of maternal origin transmitted to the second generations. Significant departures of $\hat{q}$ from 0·5 are denoted by * (5 per cent), ** (1 per cent) and *** (0·1 per cent)

		Ovary colour			Flower colour		
		$\hat{q}$	s.e.	sig.	$\hat{q}$	s.e.	sig.
20B	M_2	0·526	0·030	ns	0·495	0·032	ns
20B	BC	0·524	0·016	ns	0·517	0·012	ns
20B	RBC	0·563	0·016	***	0·508	0·012	ns
20C	M_2	0·548	0·024	ns	0·597	0·033	**
20C	BC	0·657	0·036	***	0·576	0·035	*
20C	RBC	0·502	0·029	ns	0·581	0·026	**

treatment. Whilst for a given character many M_1 plants produced at least one deviant family only a few gave significant deviations in all three families. In some cases there was a significant difference between the two backcross families indicating stronger selection through one of the two gametophytes more commonly the pollen. The effects of mutational loss of the black ovary pigment were apparent in the entirely non-black families produced from non-black 20B M_1 plants. A similar loss of one of the two alleles for the green flower colour pigment, however, gives families segregating at only one gene, a situation which cannot be distinguished from strong maternal selection. Two sets of families from 20B M_1 plants displayed deviations for ovary colour which are difficult to explain. Firstly, both backcrosses from one M_1 contained significantly more paternal alleles than expected. It is difficult, however, to envisage radiation inducing selection in favour of the irradiated chromosomes and a more likely explanation is the duplication of the chromosome or a segment of the chromosome carrying the black allele in the original male gamete. Secondly, one non-black 20B M_1 plant gave 3 black ovaried plants out of 20 in both backcross families. The original M_1 plant may have been misclassified for this character although it is also possible that it may have been mosaic for this gene, with the non-black sector being scored for the character whilst both sectors were used for the cross.

DISCUSSION

The inheritance of ovary colour following pollen irradiation demonstrates clearly the two effects the radiation may have on a character. Firstly, damage or deletion of the functional, black allele of V_{12} reduces it to a functionless form indistinguishable in effect from the functionless, non-black allele of V_{27} and leads to the production of non-black 20B M_1 plants. The effects of mutational loss are not however observed when the functionless allele is irradiated as was the case with the 20C treatment. Secondly, selection either in the M_1 gametophytes or in the resultant zygotes against mutational damage linked to this gene disturbs its segregation in the second generation regardless of the direction of the cross. Thus, an excess of non-black alleles were observed in the 20B treatment and an excess of black alleles in the 20C treatment. The same trends were apparent in the other two characters examined both here and by Caligari *et al.*, although the effects of selection and mutational loss cannot be separated in these cases. Both effects produce only small disturbances in the allele frequencies of the second generation.

If pollen irradiation is to be used for the transfer of single genes as an alternative to conventional backcrossing it is essential that the inheritance of undesirable radiation damage is negligible and that the effects of selection are strong. The results presented here and in our previous paper (Werner and Cornish, 1985) indicate that neither is the case in this material. It is interesting, therefore, to compare our findings with those of others. Mutational loss at a major gene following pollen irradiation has been reported by many workers; Zamir (1983) induced the loss of the paternal allele of an isozyme locus in tomato; Davies (1984) induced the loss of a dominant stipule marker in *Pisum sativum*; Daskalov (1984) reported the mutational loss of an allele for anthocyanin pigmentation in *Capsicum*. The loss of the dominant paternal alleles for ear morphology characters in the M_1 *Triticum aestivum* plants described by Snape *et al.* (loc. cit.) can be interpreted as conventional mutational loss as can the M_1 data for mildew resistance and growth habit reported by Powell *et al.* (1983) on *Hordeum vulgare*. Disturbed segregations of qualitative characters have also been observed by a number of groups and estimates of q can be derived from their published data to permit comparison.

Table 4 Estimates of q, calculated from published data. All control segregations follow Mendelian ratios giving $q = 0{\cdot}5$, except those marked ‡ where the appropriate control value of q is given

Source reference	Organism	Dose (Krad)	Markers used and estimates of q	Mean $\hat{q}$
Werner (1984)	*N. rustica*		flower colour, ovary colour	
		γ ray 2	0·550, 0·468	0·509
		γ ray 10	0·565, 0·506	0·535
		γ ray 20	0·546, 0·553	0·550
Ingram (1982)	*N. rustica*		flower colour, ovary colour	
		0	—, 0·573‡	
		X ray 10	0·543, 0·590	0·567
		X ray 15	0·524, 0·593	0·559
		γ ray 10	0·539, 0·561	0·550
		γ ray 15	0·551, 0·532	0·542
		γ ray 20	0·643, 0·523	0·583
Zamir (1983)	*Lycopersicon/Solanum*		electrophoretic enzymes (7)	
		γ ray 20	0·487, 0·545, 0·624, 0·538, 0·533, 0·527, 0·478	0·533
Snape *et al.* (1983)	*T. aestivum*		electrophoretic enzymes (8)	
		γ ray 2	0·769, 0·482, 0·553, 0·509, 0·500, 0·581, 0·620, 0·571	0·573
Davies (1984)	*P. sativum*		various visual markers (5)	
		X ray 0·9	0·517, 0·489, 0·525, 0·480, 0·484	0·499
		X ray 1·2	0·514, 0·496, 0·521, 0·531, 0·483	0·509
		X ray 1·8	0·488, 0·507, 0·513, 0·504, 0·507	0·504
Daskalov (1984)	*Capsicum*		various visual markers (3)	
		γ ray 1·5	0·356, 0·588, 0·640	0·528
Donini *et al.* (1970)	*H. vulgare*		Induced mutations	
		γ ray 1·5	0·533	0·533
Powell *et al.* (1983)	*H. vulgare*		mildew resist', juvenile growth habit (2)	
		0	0·503, 0·535‡, 0·389‡	
		γ ray 0·5	0·591, 0·609, 0·424	0·541
		γ ray 1·0	0·761, 0·790, 0·533	0·695
		γ ray 1·5	0·957, 0·965, 0·809	0·910
		γ ray 2·0	0·991, 1·0, 1·0	0·997

Numbers in brackets indicate the number of markers studied.

These values are given in table 4 as are the estimates from this study and additional estimates that we have obtained from lower irradiation doses (Werner, 1984). With the exception of the data of Powell *et al.* all estimates are very similar and suggest that only weak selection in favour of maternal alleles can be induced by pollen irradiation. Only the results of Powell *et al.* indicate that selection of a useful intensity can be achieved, although the reasons for the difference between their results and those of others are not at present apparent.

The frequency of transmission of maternal chromosomes to the second generation of *N. rustica* when selection acts against chromosomal aberrations was found to be 0·67 (Werner and Cornish, 1984). Since this represents selection against the most severe forms of radiation damage it is not surprising that it is more intense than the selection observed here against major genes ($q = 0{\cdot}55$), which can be taken as more typical of the average selection acting on the whole genome. As was discussed in our previous paper the frequency of maternal alleles would need to be increased to 0·82 for selection to account for the reduction in final height observed by Caligari *et al.* (loc. cit.). The effects of radiation on quantitatively inherited characters such as final height will be examined in the final paper in this series (Cornish and Werner, 1985).

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